Kidney International, Vol. 37 (1990), pp. 1316–1324

Eicosanoid synthesis in human peritoneal macrophages stimulated with *S. epidermidis*

RUTH K. MACKENZIE, GERALD A. COLES, and JOHN D. WILLIAMS

Institute of Nephrology, Kidney Research Unit Foundation, University of Wales College of Medicine, Royal Infirmary, Cardiff, CF2 1SZ, Wales, United Kingdom

Eicosanoid synthesis in human peritoneal macrophages stimulated with *S. epidermidis*. Peritoneal macrophages isolated from CAPD patients phagocytozed *S. epidermidis* in a time dependent manner. Coincident with a maximum phagocytic uptake of 56% by 12 hours, there was secretion of a significant amount of neutral protease (1.37 ± 0.2 mg [^{3}H]-casein degraded/10^6 cells, $P = 0.05$). In contrast to these delayed effects, coincubation of PMO with *S. epidermidis* resulted in a significant increase in both LTB_4 and LTC_4 synthesis above that of controls, with 6.33 ± 1.20 ng $LTB_4/10^6$ cells ($P < 0.01$) and 2.06 ± 0.68 ng $LTC_4/10^6$ cells ($P = 0.014$) being generated by three hours. The generation of these lipoxygenase products was both time and dose dependent, and the rapid production and release of the potently chemotactic LTB_4 is consistent with the observed clinical response, where a rapid influx of PMN into the peritoneal cavity occurs during episodes of peritonitis, while the generation of LTC_4 may contribute to the hyperemia and interstitial edema. In contrast, although there was a time dependent rise in cyclooxygenase product generation by unstimulated cells, a dose dependent inhibition of synthesis was clearly demonstrated when cells were incubated with bacteria, with a mean 40% reduction in generation of PGE_2 and a mean 34% reduction in TXB_2 generation ($P = 0.01$ and $P < 0.025$, respectively). It was demonstrated that the inhibition was not due to lack of available substrate and that the generation of eicosanoids was unrelated to phagocytosis, bacterial/PMO contact or bacterial surface characteristics. Instead, the observed effect of *S. epidermidis* on the PMO was attributable to a secreted bacterial product.

Continuous ambulatory peritoneal dialysis (CAPD) is an established form of treatment for patients with end-stage renal failure [1]. Despite its success, however, peritoneal infection remains the most common cause of patient morbidity and treatment failure [2–4]. Morphological studies on cells isolated from the peritoneal fluid, early during the course of an infection, demonstrate phagocytosis of bacteria by the resident macrophage population and suggest that these mononuclear phagocytes (PMO) form the first line of defence against invading microorganisms [5–7]. The exact nature, however, of the response of PMO to bacteria remains unclear.

PMO from CAPD patients have been reported as being more like elicited cells than resident macrophages [8] and it has been shown that elicited or activated macrophages synthesize increased quantities of neutral protease, a potentially damaging enzyme, when compared to resident cells [9–11]. In addition, the stimulation of PMO by both particulate and soluble agents results in the oxidative metabolism of arachidonic acid with the release of both cyclooxygenase and lipoxygenase products [12–15]. More recently, analysis of peritoneal fluid has demonstrated a rapid increase in these eicosanoids early in the course of infection [16] which may contribute to both the rapid influx of neutrophils into the peritoneal cavity, as well as the hyperemia and interstitial edema of peritonitis.

The most common infecting organism in CAPD peritonitis is *Staphylococcus epidermidis* (*S. epidermidis*) [17]. This species has been shown to be capable of producing a variety of secreted proteins, including a number of specific hemolysins [18] as well as a variety of other potentially damaging enzymes such as deoxyribonuclease, urease, gelatinase, caseinase, lysozyme and fibrinolysin [19].

The present study investigates the interactions between PMO obtained from patients undergoing CAPD and an unopsonized strain of *S. epidermidis*, previously isolated during an episode of peritonitis, and demonstrates that the interaction of bacteria and phagocyte results in the oxidative metabolism of arachidonic acid, as well as the release of neutral protease activity.

Methods

Patients

Human PMO were isolated from the PD effluent of 26 male and 8 female CAPD patients ranging in age from 26 to 80 years (65.09 ± 2.2 years). Of these 34 patients the underlying disease was glomerulonephritis in eight, renal vascular disease in six, interstitial nephritis in three and polycystic disease in two. The remaining 15 had a diagnosis of chronic renal failure of unknown etiology. All patients had been infection free for more than five weeks prior to the collection of their PMO and none had received antibiotic therapy for at least two weeks prior to inclusion in this study. None of the patients were receiving corticosteroids. No attempt was made to select patients with histories of either low or high peritonitis rates.

Peritoneal macrophages

Following an overnight dwell the empty drainage bag was pre-cooled for 10 minutes and the effluent was collected on ice to minimize adherence of PMO to the plastic. The dialysate was then drained into 50 ml sterile polypropylene centrifuge tubes at

Received for publication August 29, 1989
and in revised form December 12, 1989
Accepted for publication December 18, 1989

4°C and cells were isolated by centrifugation at 2500 × g for 35 minutes at 10°C. The supernatant was discarded, the cell pellets combined in 50 ml of cold, sterile, phosphate buffered saline (PBS) pH 7.3, (Dulbecco; Oxoid Ltd., Basingstoke, UK) and centrifuged at 1900 × g for 20 minutes at 10°C. After further washing (× 2) the cells were resuspended in 50 ml PBS. Two hundred microliters of suspension was counted using a Coulter counter, model ZM (Coulter Electronics Ltd., Luton, UK) and the cells resuspended in RPMI 1640, with L-glutamine (Gibco Ltd., Paisley, UK) containing 0.2% wt/vol bovine serum albumin (ICN Biomedicals Ltd., High Wycombe, UK) to a cell density of up to 2×10^6 PMO/ml. One milliliter aliquots of the cell suspension were added to 35 mm Nunc tissue culture grade petri dishes (Gibco Ltd.) and incubated at 37°C in a CO_2 incubator for 90 minutes. The non-adherent cells were removed by washing twice with warm Tyrodes-gelatin buffer [20], the washings combined and the number of non-adherent cells estimated using the Coulter counter. The purity of the cell preparation was estimated by staining a representative plate with Neat differential hematology stain (Guest Medical Ltd., Sevenoaks, UK). Greater than 95% of the adherent cells were found to be PMO, by morphology, in all monolayers used and in the majority of cases more than 98% of the cells were PMO. The remaining adherent cells consisted of a miscellaneous group including erythrocytes, lymphocytes and a few polymorphonuclear leukocytes and were considered unlikely to make any significant contribution to the results obtained.

Bacteria

The strain of *S. epidermidis* used was isolated from the effluent of a CAPD patient with peritonitis, stored on a Dorset Egg Medium slope (Oxoid Ltd.) and cultured at least twice for 24 hours in Nutrient Broth No. 2 (Oxoid Ltd.) before use. The overnight culture was centrifuged, washed and resuspended in Tyrodes-gelatin buffer to the required optical density (OD) measured in a Unicam SP500 Spectrophotometer (Phillips Scientific, Cambridge, UK) at 560 nM.

Phagocytosis studies

One milliliter of a suspension of *S. epidermidis* at an OD of 2 was added to plates of adherent PMO and incubated at 37°C in a CO_2 incubator for various times, up to 24 hours. At the end of each incubation period the bacterial suspension was tipped off and the plates were washed three times with PBS and stained with freshly made 0.01% Acridine Orange (BDH Ltd., Poole, UK) wt/vol in 0.9% saline for 45 seconds. The plates were rinsed with PBS, mounted, and examined immediately under UV light with a Leitz Dialux 20 microscope (Leitz Instruments Ltd., Luton, UK). At least 100 PMO were examined on each plate and the percentage phagocytosis was calculated, based on the number of cells ingesting two or more bacteria.

Neutral protease activity

Neutral protease activity was measured by the ability of both supernatants and cell extracts to degrade a known amount of [^{3}H]-casein, according to the method described by Steadman et al [21]. PMO were stimulated for various times with *S. epidermidis*, OD 2, for up to 24 hours, the supernatant was removed, centrifuged at 11,000 × g to remove bacteria and detached PMO and stored at −20°C. The remaining cell pellet was resuspended in 1 ml of 0.1% Triton X-100 (vol/vol; BDH Ltd.) and this was added back to the adherent cells. The plates were incubated at room temperature for 30 minutes and the lysed cells removed by scraping. The resulting debris was centrifuged and this supernatant (cell extract) was also assayed for neutral protease activity.

From the resulting counts the total (cell extract + supernatant) amount of [^{3}H]-casein degraded was calculated and expressed as mg/10^6 PMO. Cell viability was estimated in paired plates using 0.01% Acridine Orange, as detailed above.

Eicosanoid generation

Leukotriene B_4 (LTB_4), leukotriene C_4 (LTC_4), prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-K-$PGF_{1\alpha}$) were measured by specific radioimmunoassay. All [^{3}H] tracers were obtained from Amersham International plc (Aylesbury, UK). LTB_4 and LTC_4 standards were a gift of Dr. Berndt Spur (Institute Henri Beaufour, Paris, France) and antibodies to LTB_4 and LTC_4 were donated by Merck Frosst, Dorval, Canada. Prostaglandin standards and arachidonic acid were obtained from Sigma Chemical Co. Ltd. (Poole, UK) and anti-prostaglandin antibodies from Bioclinical Services (Cardiff, UK).

Monolayers of PMO were incubated with increasing concentrations of *S. epidermidis*, for different times and the generated eicosanoids, as measured by RIA were compared to the synthesis of product by paired control cultures. The cell layers were extracted at −20°C overnight in 1 ml of 100% methanol which was then removed, centrifuged, dried down in a Univap rotary vacuum evaporator (Uniscience Ltd., London, UK) and reconstituted in Tris-isogel buffer containing 0.1 M Tris-HCl, 0.9% NaCl (wt/vol) and 0.1% gelatin (wt/vol; Difco Laboratories Ltd., East Molesey, UK) [20], pH 7.4, before assaying.

Eicosanoids were assayed in duplicate, by radioimmunoassay (RIA) in Tris-isogel buffer, pH 7.4. One hundred microliters of sample or standard, 100 μl of antibody and 100 μl of [^{3}H]-tracer were incubated in polypropylene tubes (Luckham Ltd., Burgess Hill, UK) at 4°C overnight for LTC_4, PGE_2 and TXB_2 and at 37°C overnight for LTB_4. Bound and free eicosanoids were separated by addition of 200 μl of cold dextran T70 (Pharmacia-LKB Ltd., Milton Keynes, UK) coated charcoal (Norit SX-1; BDH Ltd.), both 1% wt/vol in Tris-isogel buffer, and centrifuged immediately for 10 minutes at 4000 rpm at 4°C. Supernatants were decanted, mixed with 3.5 ml Optiphase MP scintillant (Pharmacia-LKB Ltd.) and counted for two minutes in an LKB β counter. LTB_4 and LTC_4 were measured over the linear part of the curve from 0.1 to 4 ng/ml while PGE_2 and TXB_2 were measured over the linear part of the curve from 0.8 to 2.5 ng/ml.

Eicosanoid generation was measured on at least three separate occasions using cells from different donors. All experiments were performed in parallel with paired control PMO monolayers, using cells from the same donors.

The viability of the PMO monolayers, co-incubated with *S. epidermidis*, was assessed on a representative plate after three hours at concentrations of bacteria of OD = 0.5 and OD = 2, run in parallel with the three dose-response experiments. Cell viability was estimated using 0.01% Acridine Orange, as previously described.

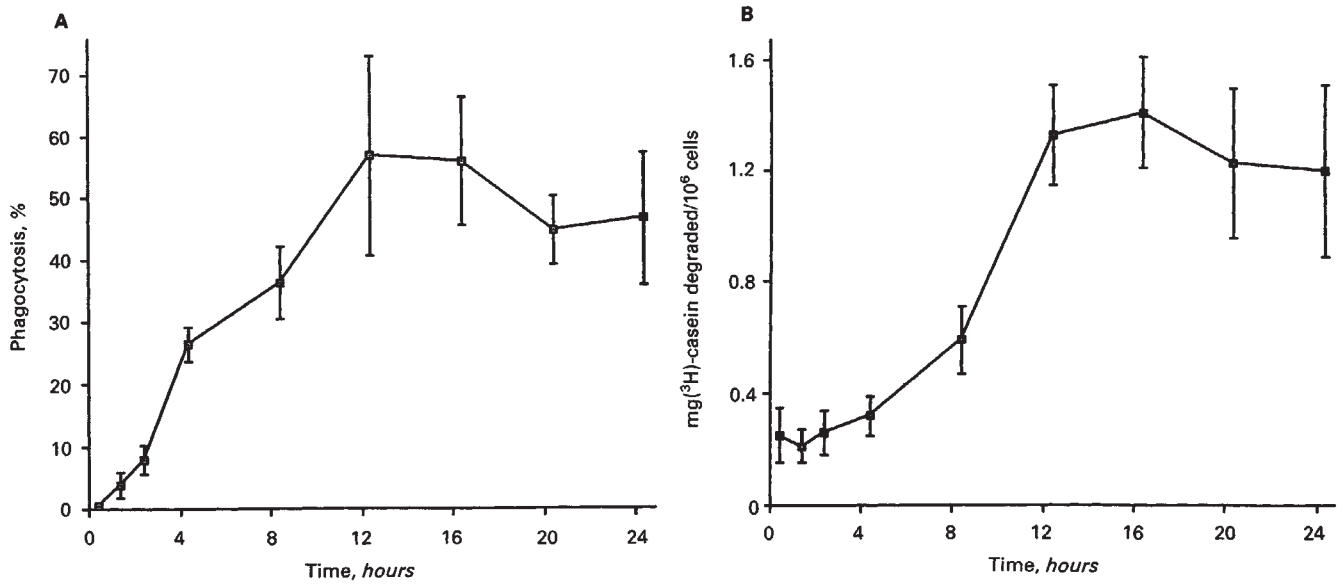

Fig. 1. A. *Time dependent phagocytosis of* S. epidermidis *at a concentration of OD 2 by PMO monolayers.* The results are expressed as the mean % of organisms ingesting 2 or more bacteria/cell. **B.** Time course of release of neutral protease activity (mg[^{3}H]-casein degraded/10^6 cells) from PMO monolayers incubated with *S. epidermidis* at a concentration of OD 2. The results are expressed as the mean ± SEM for 3 separate experiments using cells from different donors.

Preparation of killed bacteria

Cultures of *S. epidermidis* were killed either by formalin treatment or by heating, as previously described for *Escherichia coli* [22]. The killed organisms were then thoroughly washed with Tyrodes-gelatin buffer, resuspended to the required OD in this buffer and used in the experiments in the same manner as live bacteria. All preparations of killed bacteria were tested for viable organisms by culturing 100 μl of the preparation on nutrient agar (Oxoid Ltd.), overnight at 37°C.

Preparation of bacteria-free supernatants

Bacteria-free supernatants were prepared by resuspending *S. epidermidis* for three hours at 37°C in Tyrodes-gelatin buffer to an OD of 0.5. The organisms were then removed by centrifugation at 1900 × g for 20 minutes at 4°C and the resulting bacteria-free supernatant cultured with PMO in the usual manner. Supernatants were free of live bacteria as assessed following growth on agar for 18 hours at 37°C and contained no detectable LTB_4 as measured by specific RIA.

Statistics

The results were compared to control values using a Mann-Whitney U test, and the figures quoted are the mean value ± standard error of the mean (SEM).

Results

Phagocytosis/neutral protease release

PMO ingested unopsonized *S. epidermidis*, in a time dependent manner. Phagocytosis, at a bacterial concentration of 2 × 10^8 cfu/ml (OD 2), progressed slowly with <10% of PMO showing uptake of organisms by two hours. By four hours, however, a significant proportion of PMO (>25%) had ingested two or more organisms. Ingestion of microorganisms continued to rise reaching a mean of 56% by 12 to 16 hours (Fig. 1A).

The total measurable neutral protease activity also increased over the same time period, achieving significance ($P = 0.05$) over control incubations by 12 hours. Greater than 75% of the total enzyme measured was found in the supernatant. The peak levels of neutral protease activity coincided with peak phagocytosis at 12 to 16 hours with a mean 1.37 ± 0.2 mg [^{3}H]-casein being degraded/10^6 cells (Fig. 1B). There was no synthesis of neutral protease by unstimulated cells over the same time period. Incubation of *S. epidermidis* in buffer alone did not yield measurable neutral protease activity either in the supernatant or in the Triton extracted bacteria. There was no correlation between neutral protease release and cell death (results not shown).

Background eicosanoid generation

The total (intracellular + extracellular) generation of cyclooxygenase products by unstimulated, adherent PMO ($N = 3$) was studied over a time course of three hours in Tyrodes/gelatin buffer (Fig. 2). The levels of PGE_2 and TXB_2 rose steadily over this time period with levels of PGE_2 reaching 12.89 ± 0.91 ng/10^6 cells and for TXB_2 were 30.87 ± 9.47 ng/10^6 cells. Further incubation of cells, for times up to 24 hours, showed a continued rise of PGE_2, up to eight hours but no change in the levels of TXB_2. In contrast, there was very little generation of either LTB_4 or LTC_4, with release of both these products reaching peak concentrations for LTC_4 of 1.64 ± 1.20 ng/10^6 cells and for LTB_4 of 1.60 ± 0.84 ng/10^6 cells after two hours of incubation.

Generation of lipoxygenase products in response to stimulation with S. epidermidis

Immunoreactive LTB_4 and LTC_4 were generated by PMO, co-incubated with *S. epidermidis*, at an OD = 0.5, in a time dependent manner (Fig. 3A). After a three hour incubation

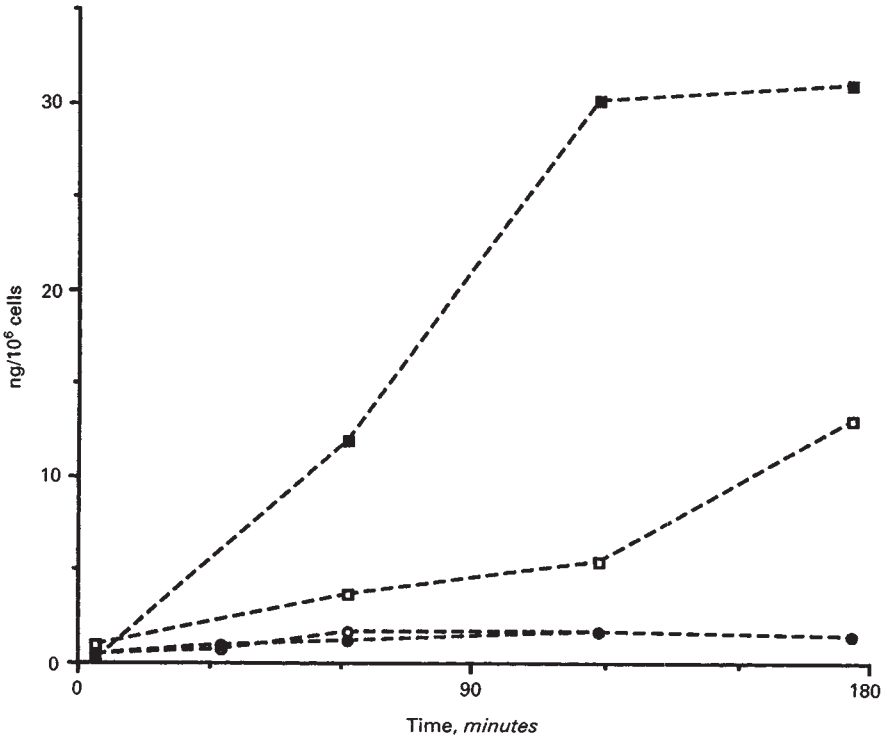

Fig. 2. *Time dependent generation of LTB_4 (●), LTC_4 (○), TXB_2 (■) and PGE_2 (□) by PMO monolayers cultured in the absence of exogenous stimuli.* Results are expressed as the mean for 3 separate experiments, using cells from different donors.

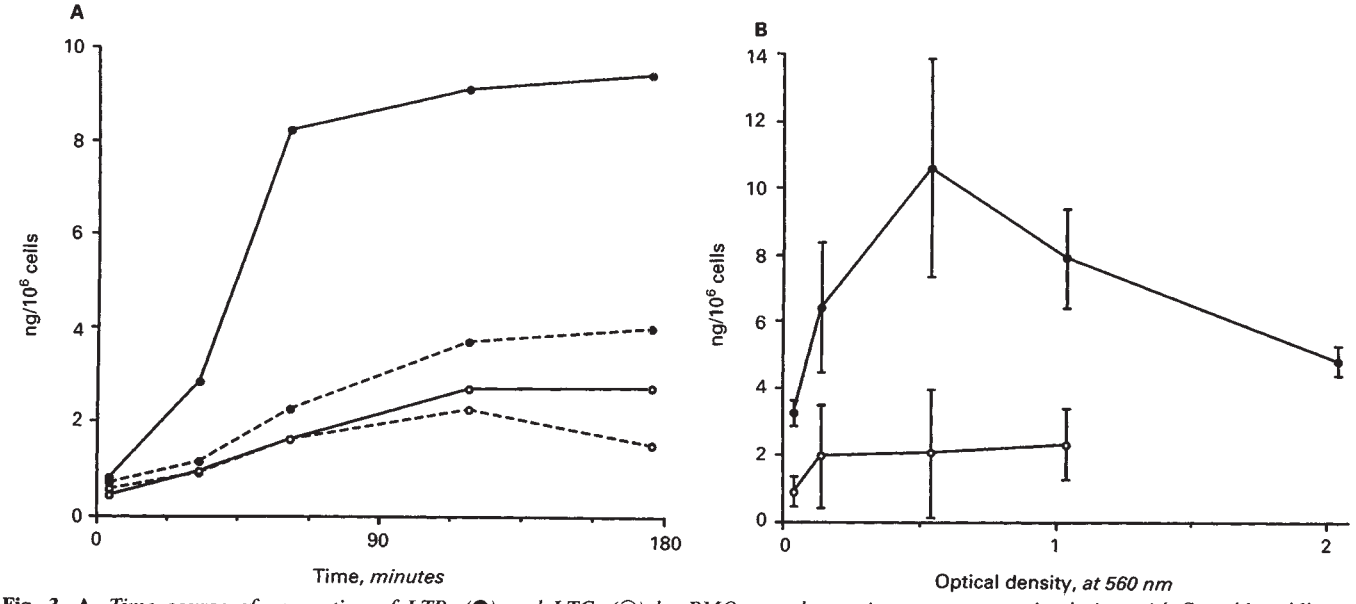

Fig. 3. A. *Time course of generation of LTB_4 (●) and LTC_4 (○) by PMO monolayers in response to stimulation with* S. epidermidis *at a concentration of OD 0.5 (——) or in control cells (----).* Results are expressed as the mean for 4 experiments, using cells from different donors. **B.** Dose dependent release of LTB_4 (●) and LTC_4 (○) by PMO monolayers in response to incubation with *S. epidermidis* for 3 hr at 37°C. Results are expressed as the mean ± SEM for 3 experiments using cells from different donors.

>75% of the LTB_4 and >50% of the LTC_4 generated was released into the culture supernatant. The total generated (intracellular + extracellular) LTB_4 reached a plateau after 60 minutes of incubation while the levels of LTC_4 continued to rise up to two hours following stimulation. The synthesis of LTB_4 was also dose dependent reaching an optimum generation when PMO were incubated with bacteria at a concentration of OD 0.5. The generation of LTC_4 showed a two-fold increase when cells were incubated with bacteria at a concentration of OD 0.1 and remained constant at higher bacterial concentrations (Fig. 3B).

In 18 experiments where total synthesized LTB_4 was measured at three hours after incubation of PMO with *S. epidermidis* OD 0.5 (Fig. 5), the stimulated cells showed a significant rise

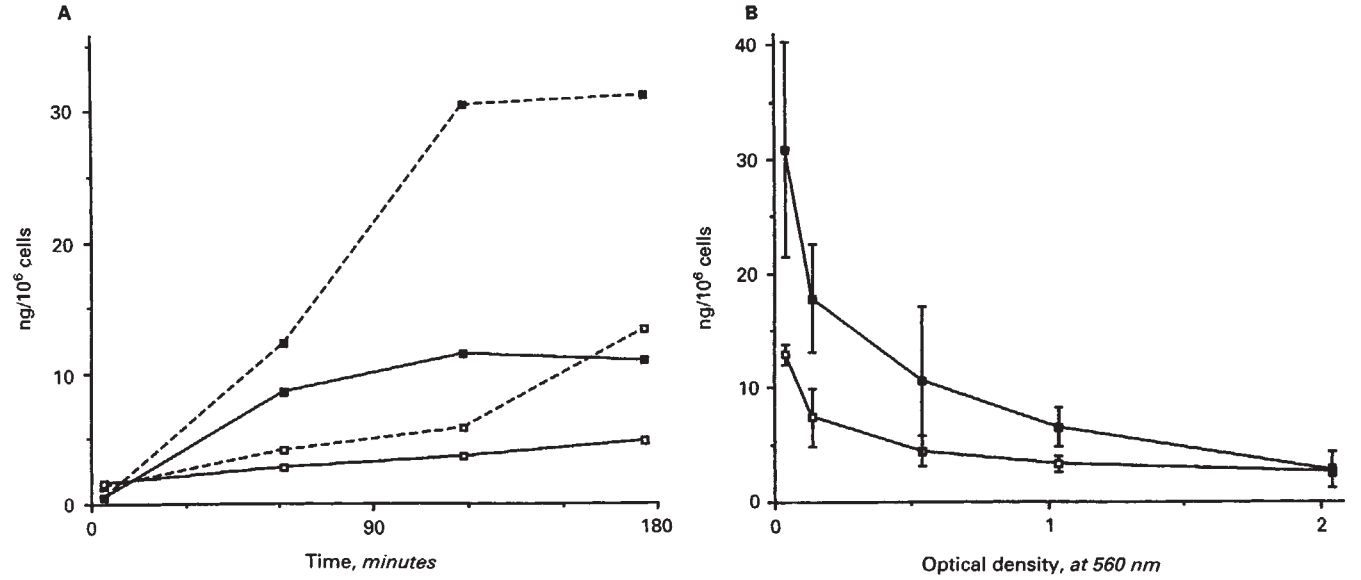

Fig. 4. A. *Time course of generation of TXB_2 (■) and PGE_2 (□) by PMO monolayers in response to stimulation with* S. epidermidis *at a concentration of OD 0.5 (——) or in cells incubated in medium alone (----).* Results are expressed as the mean for 3 experiments, using cells from different donors. **B.** Dose dependent release of TXB_2 (■) and PGE_2 (□) by PMO monolayers in response to incubation with *S. epidermidis* for 3 hr at 37°C. Results are expressed as the mean for 3 experiments, using cells from different donors.

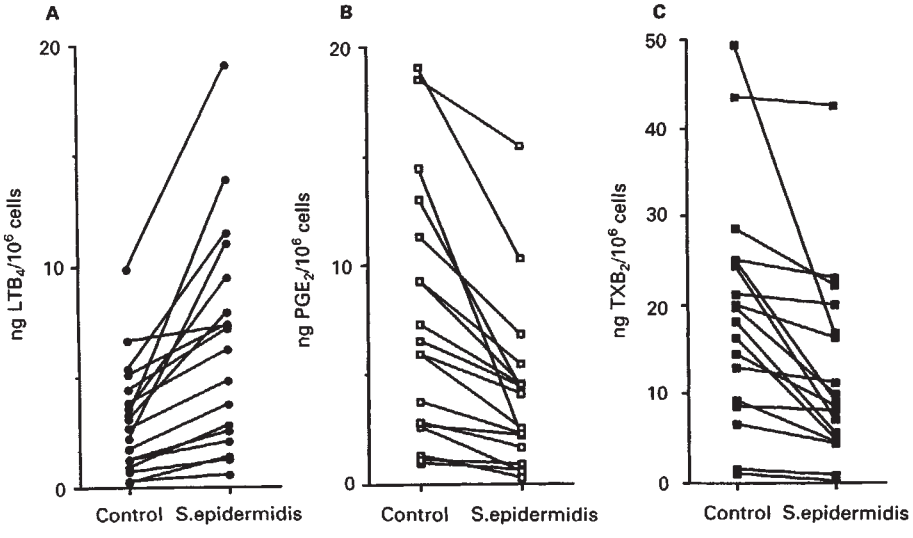

Fig. 5. *Variability in response to stimulation with* S. epidermidis *by monolayers of isolated PMO for LTB_4 (●), PGE_2 (□) and TXB_2 (■),* N = *18.*

above control levels with values of 6.33 ± 1.20 ng/10^6 cells compared to 3.07 ± 0.63 ng/10^6 cells, respectively ($P < 0.01$). In eight experiments where the level of LTC_4 was measured under the same conditions, as detailed above, the amount generated by stimulated cells (2.06 ± 0.68 ng/10^6 cells) was significantly raised above that of control cells (0.89 ± 0.52 ng/10^6 cells; $P = 0.014$).

Further incubation of cells for times up to 24 hours demonstrated a modest fall in LTB_4 levels in stimulated cells with 3.11 ± 1.54 ng/10^6 cells being measurable at two hours compared to 2.52 ± 1.18 ng/10^6 cells measured at 24 hours ($N = 6$).

Generation of cyclooxygenase products in response to stimulation with S. epidermidis

There was a time dependent increase in each of the cyclooxygenase products measured (Fig. 4A) but in comparison with paired controls, an inhibition of production of PGE_2 and TXB_2 was clearly demonstrated when PMO were incubated with bacteria at a concentration of OD 0.5. An inhibition of generation of cyclooxygenase products was also demonstrated when the dose response of the PMO to *S. epidermidis* was examined (Fig. 4B).

In 18 experiments in which PGE_2 and TXB_2 were measured

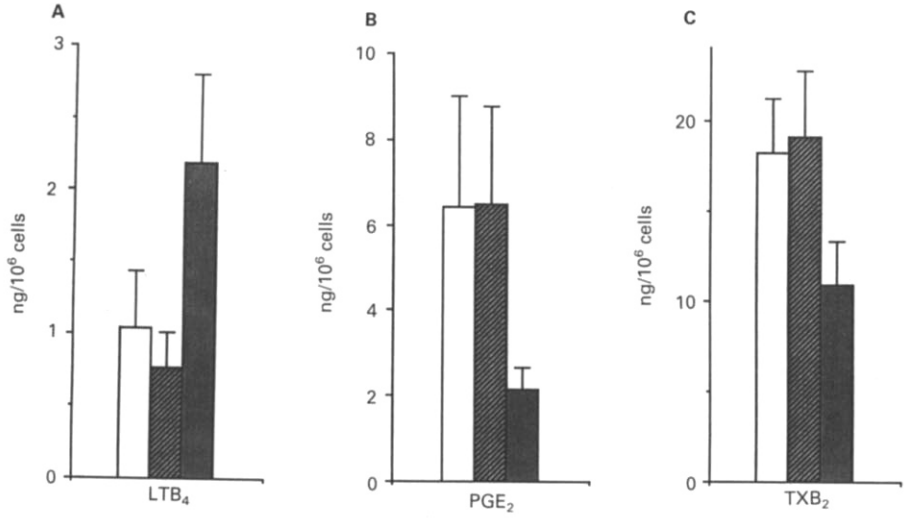

Fig. 6. *Generation of eicosanoids by PMO monolayers in response to incubation for 2 hr at 37°C with killed (▨) or live (▩) S. epidermidis at a concentration of OD 0.5 or in cells incubated in medium alone (□).* Results are expressed as the mean + SEM for 6 experiments, using cells from different donors.

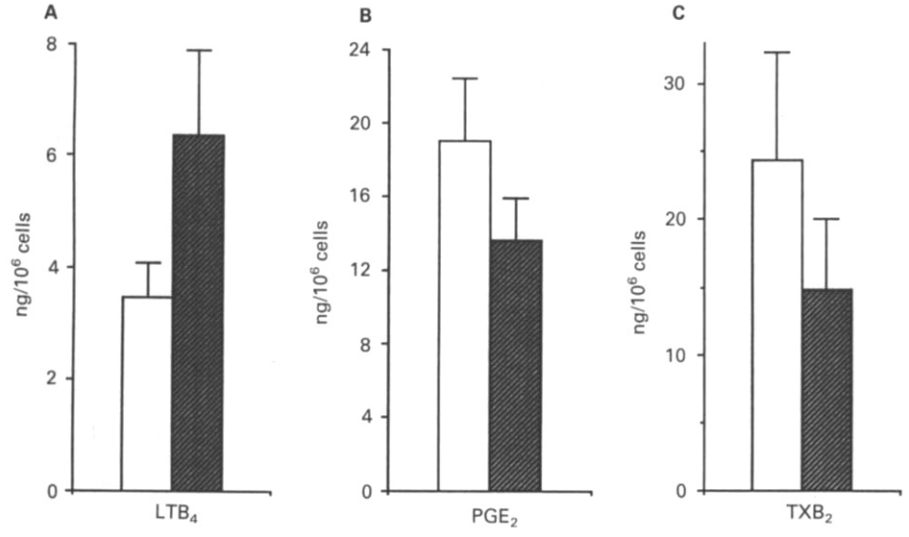

Fig. 7. *Generation of eicosanoids by PMO monolayers in response to incubation for 3 hr at 37°C in bacteria free supernatant (▨) or by cells incubated in medium alone (□).* Results are expressed as the mean + SEM for 5 experiments, using cells from different donors.

after three hours of incubation with *S. epidermidis* at a concentration of OD 0.5 (Fig. 5), there was a significant inhibition of both products with 8.86 ± 1.23 ng $PGE_2/10^6$ cells and 20.54 ± 2.65 ng $TXB_2/10^6$ cells generated by the controls, while 5.29 ± 1.02 ng $PGE_2/10^6$ cells and 13.58 ± 2.37 ng $TXB_2/10^6$ cells were generated by the PMO incubated with bacteria ($P = 0.01$ and $P < 0.025$, respectively).

Variability

A wide variability in response was noted, between cells from different donors, in all the eicosanoids measured. This variability is demonstrated in Figure 5.

Viability

In the three experiments in which cell viability was assessed following coincubation with *S. epidermidis*, it was found that, at a concentration of bacteria of OD = 0.5, 97.18 ± 2.13% of the cells remained viable after three hours, whereas at a bacterial concentration of OD = 2, 91.8 ± 4.5% cells were viable.

Eicosanoid generation in PMO stimulated with killed bacteria

Figure 6 shows the generation of eicosanoids by control PMO and paired cells stimulated with either killed or live bacteria at an OD 0.5 for three hours. Since there was no difference between the results obtained when bacteria were killed by formalin treatment or heating, the data from both sets of experiments have been combined for statistical analysis. There was no significant difference between the control PMO generation of LTB_4, PGE_2 or TXB_2 and the generation of these eicosanoids by PMO stimulated with killed bacteria. However, there was a significant increase in LTB_4 generation when PMO were stimulated with live *S. epidermidis* ($P = 0.047$). Similarly, only the live organism caused a significant inhibition in synthesis of PGE_2 and TXB_2 ($P = 0.047$).

Eicosanoid generation in PMO stimulated with bacteria free supernatants

The coincubation of bacteria-free supernatants with PMO over three hours compared to paired controls demonstrated a

similar pattern to that observed with live *S. epidermidis* (Fig. 7). The generation of LTB_4 was significantly increased ($P = 0.024$) and both TXB_2 and PGE_2 generation were significantly inhibited ($P = 0.037$; $N = 6$).

Effect of addition of exogenous substrate

Arachidonic acid was added exogenously to both control and stimulated PMO in a range of concentrations from 0 to 30 μM, concurrently with the stimulus. The resulting generation of eicosanoids by three hours after stimulation was then measured (Fig. 8). *S. epidermidis* at an OD 0.5 again stimulated LTB_4 production which was augmented at the highest concentration of arachidonic acid. The addition of the substrate did not reverse the down-regulation of PGE_2 or TXB_2 production.

Discussion

This study represents the first detailed investigation of the interaction of human PMO with unopsonized *S. epidermidis*. The phagocytosis of *S. epidermidis* by PMO has previously been reported [23] but the organisms used were opsonized, although the levels of opsonins present in CAPD effluents are very low [24]. The phagocytosis of unopsonized *S. epidermidis*, reported here, demonstrated less than a 10% uptake by PMO over the first two hours of incubation. Although by 12 hours phagocytosis had risen to a mean of 56%, this relatively slow phagocytic mechanism is unlikely to be of any clinical benefit, other than during the latter part of an overnight dwell of PD fluid in the peritoneal cavity.

The finding that PMO incubated with *S. epidermidis* secrete a neutral protease has not previously been reported, although other studies [9–11] have demonstrated the increased secretion of various neutral proteases by elicited or activated macrophages after stimulation with other ligands. It would appear, from our results, that the neutral protease is secreted in parallel with an increase in phagocytic activity, as levels of this enzyme do not rise significantly until there is greater than 56% phagocytosis. The exact significance of the secretion of this enzyme remains uncertain, though it may assist extracellular killing of microorganisms. It could also, in part, occur from destruction of the PMO by ingested bacteria, which are not always killed intracellularly [23], but we found no correlation between cell death and enzyme release. Once released into the peritoneal cavity, the protease may be capable of damaging the mesothelium and underlying connective tissue, a condition often seen following severe peritonitis [25].

It has previously been reported that PMO, from both human and animal sources, stimulated with various ligands, including zymosan and calcium ionophore, demonstrate an increased production of lipoxygenase products [14, 15, 26–29]. In addition, phagocytes from different individuals demonstrate a wide variation in eicosanoid generation in response to the same stimulus [30]. This observation has been confirmed in the present study (Fig. 5) and to take account of this variability all experiments were performed with paired controls. Our results demonstrate that using an isolate of *S. epidermidis*, a significant generation and release of both LTB_4 and LTC_4 occurs in a dose and time dependent manner. The concentration of bacteria, required for the optimum production of LTB_4, was at an OD = 0.5 and there was a subsequent decrease when higher concentrations of *S. epidermidis* were used. This apparent fall in production may, in part, be due to cell death, when high bacterial concentrations were used (a mean 2.82% cell death at OD = 0.5 and a mean 8.2% cell death at an OD = 2, after 3 hr incubation) but it is unlikely to account for the whole of the mean 54.6% drop in measurable LTB_4. This decrease may instead be as the result of metabolism of LTB_4 to a non-immunoreactive dihydro product [31]. These results suggest that the production of the potent chemotactic LTB_4 by the PMO, within one hour of stimulation, may induce a rapid influx of polymorphonuclear leucocytes (PMN) into the peritoneal cavity, a factor consistent with the observed clinical response

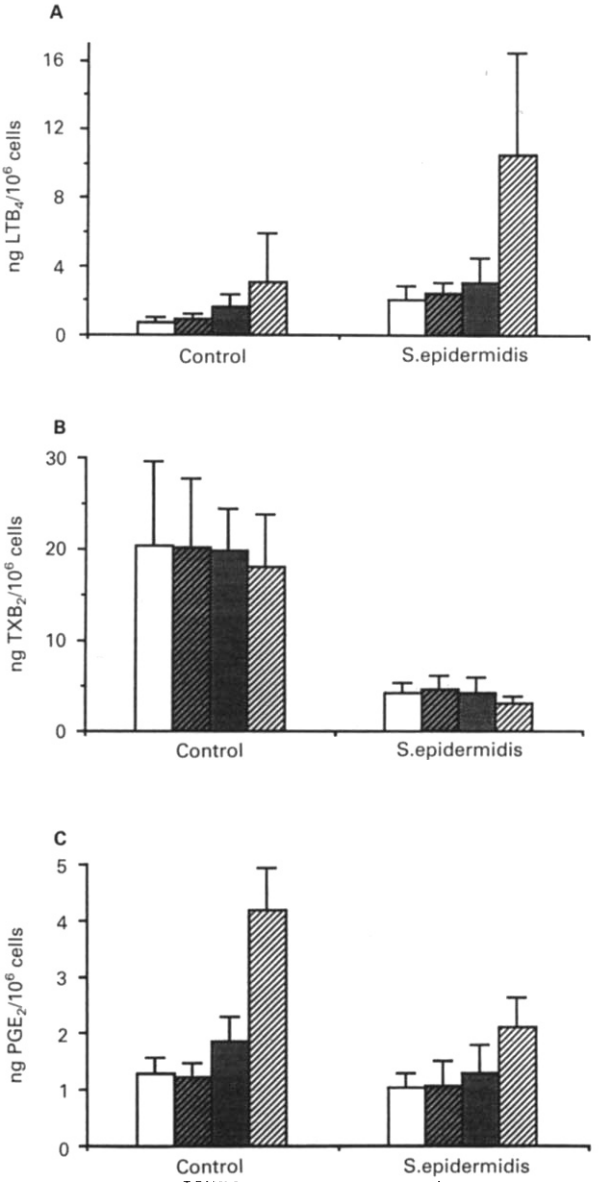

Fig. 8. *Generation of eicosanoids,* **(A)** *LTB_4,* **(B)** *PGE_2 and* **(C)** *TXB_2 by PMO monolayers in response to incubation for 3 hr at 37°C with* S. epidermidis *or in cells incubated in medium alone.* Incubations were carried out in the presence of increasing concentrations of exogenous arachidonic acid (□ no arachidonic acid, ▨ 0.3 μM arachidonic acid, ▩ 3 μM arachidonic acid, ▨ 30 μM arachidonic acid). Results are expressed as the mean + SEM for 3 experiments, using cells from different donors.

seen during episodes of peritonitis, and that the generation of LTC_4 may contribute to the hyperemia and interstitial edema of peritonitis. An increase in LTB_4 has also been shown to augment the release of lysosomal enzymes from PMN [32], but the much delayed release of neutral protease from PMO in our studies would suggest that no such autocrine action occurs with the PMO.

The present study also demonstrates a specific down regulation of the synthesis of cyclooxygenase pathway products in response to stimulation with *S. epidermidis*. This finding is consistent with the demonstration by Fieren and colleagues [33, 34] that PMO isolated from the peritoneal cavities of patients during episodes of peritonitis demonstrated a marked decrease in cyclooxygenase activity. In contrast, studies on murine macrophages elicited by *Listeria monocytogenes* showed a selective down-regulation of both lipoxygease and cyclooxygenase products with preservation of only the thromboxane synthase activity [35].

It has been shown that E-type prostaglandins inhibit or suppress diverse effector systems of inflammation [8, 33, 34, 36] suggesting that they may have anti-inflammatory actions. Furthermore, they decrease lysosomal hydrolase release, locomotion and phagocytosis by rodent macrophages, and these effects would be deterimental to the clearance of infection. Thus, the observed down-regulation of PGE_2 production by human PMO, when stimulated with *S. epidermidis* could be beneficial to the clearance of the microorganisms.

It has previously been suggested that substrate availability is crucial to the simultaneous generation of products of both the lipoxygenase and cyclooxygenase pathways. Humes and colleagues [37] have demonstrated decreased deacylation of cellular phospholipids, in elicited mouse PMO, and a marked decrease in the release of ^3H-arachidonic acid when compared to non-elicited cells. In the present study we failed to demonstrate recovery of cyclooxygenase product generation following the addition of exogenous arachidonic acid. In contrast, the production of LTB_4 alone was enhanced by the higher concentrations of substrate. These results would appear to indicate that the inhibition of the cyclooxygenase pathway is not due to the lack of availability of substrate and that the lipoxygenase pathway is preferentially selected in these cells.

The kinetics of phagocytosis and enzyme release that we have demonstrated show that by two hours, less than 10% of the PMO had ingested bacteria and there was no increase in neutral protease release. In contrast, the generation of leukotrienes by these stimulated cells had already reached a maximal level of synthesis. This would suggest that either direct PMO/bacterial contact or a secreted bacterial product may be responsible for the observed eicosanoid generation. If the response was due to cell/cell contact, the implication would be that the cell wall constituents of the *S. epidermidis* were responsible for eliciting this response. There was, however, no increase in leukotriene generation nor any inhibition of cyclooxygenase products in response to killed bacteria. In contrast, the finding that the response to live bacteria could be reproduced using a bacteria-free supernatant would implicate a bacterial product. Bacteria secrete a wide variety of hemolysins or toxins and enzymes [17, 18] which may have damaging effects against other cell types. They may cause membrane damage, be cytotoxic, cytolytic, cause dermonecrosis or may even be lethal when injected intravenously. In addition, alpha toxin, secreted by *S. aureus* has been shown to stimulate LTB_4 production by rabbit PMN [38]. The exact nature and mechanism of action of the secreted product of *S. epidermidis*, responsible for eliciting the responses demonstrated in this paper, is currently under investigation in our laboratory.

In conclusion, PMO obtained from CAPD patients respond to stimulation by *S. epidermidis* by increased generation of leukotrienes and decreased production of cyclooxygenase products. This effect is independent of phagocytosis and is probably initiated by a secreted bacterial product. These responses of PMO probably contribute to the early phase of peritonitis and are likely to be important in host defense during CAPD treatment.

Acknowledgments

LTB_4 and LTC_4 used in this study were gifts from Dr. Berndt Spur, Institute Henri Beaufour, Paris, France, and antibodies to LTB_4 and LTC_4 were gifts of Merck Frosst, Dorval, Canada. We thank the staff of the CAPD Unit at Cardiff Royal Infirmary for all their cooperation and help in obtaining suitable donors and in the collection of PD effluent.

Reprint requests to Dr. J.D. Williams, Institute of Nephrology, University of Wales College of Medicine, Royal Infirmary, Cardiff CF2 1SZ, Wales, United Kingdom.

References

1. Gokal R: Historical development and clinical use of continuous ambulatory peritoneal dialysis, in *Continuous Ambulatory Peritoneal Dialysis*, edited by Gokal R, Edinburgh, Churchill Livingstone, 1986, pp. 1–13
2. Smith WGJ, Tsakiris DJ, Junor BJR, Briggs JD, Alcock SR, Reading P, Hallam S: Peritonitis in continuous ambulatory peritoneal dialysis. *Scott Med J* 31:85–89, 1986
3. Gloor HJ, Nichols WK, Sorkin MI, Prowant BF, Kennedy JM, Baker B, Nolph KD: Peritoneal access and related complications in continuous ambulatory peritoneal dialysis. *Am J Med* 74:593–598, 1983
4. Kurtz SB, Wang VH, Anderson CF, Vogel JP, McCarthy JT, Mitchell JC, Kuman R, Johnson WJ: Continuous ambulatory peritoneal dialysis. Three years experience at the Mayo Clinic. *Mayo Clinic Proc* 58:633–689, 1983
5. Khanna R: Thoughts on the future of continuous ambulatory peritoneal dialysis. *Perit Dial Bull* 1:78–81, 1981
6. Dunn DL, Barke RA, Ewald DC, Simmons RL: Macrophages and translymphatic absorption represent the first line of host defence of the peritoneal cavity. *Arch Surg* 122:105–110, 1987
7. Goldstein CS, Bomalaski JS, Zurier RB, Neilson EG, Douglas SD: Analysis of peritoneal macrophages in continuous ambulatory peritoneal dialysis patients. *Kidney Int* 26:733–740, 1984
8. Bonta IL, Adolfs MJP, Fieren WJA: Cyclic AMP levels and their regulation by prostaglandins in peritoneal macrophages of rats and humans. *Int J Immunopharmacol* 6:547–555, 1984
9. Unkeless JC, Gordon S, Reich E: Secretion of plasminogen activator by stimulated macrophages. *J Exp Med* 139:834–850, 1974
10. Werb Z, Gordon S: Secretion of a specific collagenase by stimulated macrophages. *J Exp Med* 142:346–360, 1975
11. Werb Z, Gordon S: Elastase secretion by stimulated macrophages. *J Exp Med* 142:361–377, 1975
12. Foegh M, Maddox YT, Winchester J, Rakowski T, Schreiner G, Ramwell PW: Prostacyclin and thromboxane release from human peritoneal macrophages. *Adv Prostaglandin Thromboxane Leukotriene Res* 12:45–49, 1983
13. Scott WA, Zrike JM, Hamill AL, Kempe J, Cohn ZA: Regulation of arachidonic acid metabolites in macrophages. *J Exp Med* 152:324–335, 1980
14. Laviolettte M, Carreau M, Coulombe R, Cloutier D, Dupont P, Rioux J, Braquet P, Borgeat P: Metabolism of arachi-

donic acid through the 5-lipoxygenase pathway in normal human peritoneal macrophages. *J Immunol* 141:2104–2109, 1988
15. DU JT, FOEGH M, MADDOX Y, RAMWELL PW: Human peritoneal macrophages synthesize leukotrienes B4 and C4. *Biochim Biophys Acta* 753:159–163, 1983
16. STEINHAUER HB, GUNTER B, SCHOLLMEYER P: Stimulation of peritoneal synthesis of vasoactive prostaglandins during peritonitis in patients on continuous ambulatory peritoneal dialysis. *Eur J Clin Invest* 15:1–5, 1985
17. MION C, SLINGENEYER A, CANAUD B: Peritonitis, in *Continuous Ambulatory Peritoneal Dialysis*, edited by GOKAL R, Edinburgh, Churchill Livingston, 1986, pp. 163–217
18. MÖLLBY R: Isolation and properties of membrane damaging toxins, in *Staphylococci and Staphylococcal Infections*, edited by EASMON CSF, ADLAM C, London, Academic Press, 1983, vol. 2, pp. 619–669
19. GEMMELL CG: Extracellular toxins and enzymes of coagulase-negative staphylococci, in *Staphylococci and Staphylococcal Infections*, edited by EASMON CSF, ADLAM C, London, Academic Press, 1983, vol. 2, pp. 809–827
20. WILLIAMS JD, LEE TH, LEWIS RA, AUSTEN KF: Intracellular retention of the 5-lipoxygenase pathway product, leukotriene B4, by human neutrophils activated with unopsonized zymosan. *J Immunol* 134:2624–2630, 1985
21. STEADMAN R, TOPLEY N, JENNER DE, DAVIES M, WILLIAMS JD: Type 1 fimbriate *Escherichia coli* stimulates a unique pattern of degranulation by human polymorphonuclear leukocytes. *Infect Immun* 56:815–822, 1988
22. RADFORD NJ, CHICK S, LING R, COLES GA, ASSCHER AW: The effect of active immunisation on ascending pyelonephritis in the rat. *J Pathol* 112:169–175, 1974
23. MCGREGOR SJ, BROCK JH, BRIGGS JD, JUNOR BJR: Bactercidial activity of peritoneal macrophages from continuous ambulatory dialysis patients. *Nephrol Dial Transpl* 2:104–108, 1987
24. VERBRUGH HA, KEANE WF, HOIDAL JR, FREIBERG MR, ELLIOT GR, PETERSON PK: Peritoneal macrophages and opsonins: Antibacterial defense in patients undergoing chronic peritoneal dialysis. *J Infect Dis* 147:1018–1029, 1983
25. GANDHI VA, HUMAYUN HM, ING TS, DAUGIRDAS JT, JABLOKOW VR, IWATSUKI S, GEIS WP, HANO JE: Sclerotic thickening of the peritoneal membrane in maintenance peritoneal dialysis patients. *Arch Intern Med* 140:1201–1203, 1980
26. ZIJLSTRA FJ, VAN DEN BROEK AMWC, VINCENT JE, DIDERICH PPHM, OP DE HOEK-FES AMC, CLAEYS M: Formation of leukotriene B4, 20-hydroxy leukotriene B4 and other arachidonic acid metabolites by macrophages during peritonitis in patients with continuous ambulatory peritoneal dialysis. *Prostaglandin Leukotriene Med* 27:151–160, 1987
27. OUWENDIJK RJTH, ZIJLSTRA FJ, VAN DEN BROEK AMWC, BROUWER A, WILSON JHP, VINCENT JE: Comparison of the production of eicosanoids by human and rat peritoneal macrophages and rat Kupffer cells. *Prostaglandins* 35:437–446, 1988
28. BONNEY RJ, HUMES JL: Physiological and pharmacological regulation of prostaglandin and leukotriene production by macrophages. *J Leukocyte Biol* 35:1–10, 1984
29. ROUZER CA, SCOTT WA, COHN ZA, BLACKBURN P, MANNING JM: Mouse peritoneal macrophages release leukotriene C in response to a phagocytic stimulus. *Proc Natl Acad Sci USA* 77:4928–4932, 1980
30. TOPLEY N, STEADMAN R, PETERSEN MM, SPUR B, WILLIAMS JD: Phagocytosis, the respiratory burst and the 5-lipoxygenase pathway are independently regulated in human polymorphonuclear leukocytes. *Adv Biosci* 66:123–129, 1987
31. SCHÖNFELD W, SCHLÜTER B, HITGER R, KÖNIG W: Leukotriene generation and metabolism in isolated human lung macrophages. *Immunology* 64:529–536, 1988
32. HAFSTROM I, PALMBLAD J, MALMSTEN CL, RADMARK O, SAMUELSSON B: Leukotriene B4—a sterospecific stimulator for release of lysosomal enzymes from neutrophils. *FEBS Lett* 130:146–148, 1981
33. FIEREN MWJA, ADOLFS MJP, BONTA IL: Alterations in sensitivity and secretion of prostaglandins of human macrophages during CAPD-related peritonitis, in *Contributions to Nephrology*, edited by BERLYNE GM, GIOVANNETTI S, Basel, Karger, 1987, vol. 57, pp. 55–62
34. ADOLFS MJP, FIEREN MWJA, BONTA IL: Infectious-inflammatory changes in cyclic AMP levels and in their regulation by prostaglandins in human peritoneal macrophages. *Prostaglandin Leukotriene Med* 18:217–226, 1985
35. TRIPP CS, LEAHY KM, NEEDLEMAN P: Thromboxane synthase is preferentially conserved in activated mouse peritoneal macrophages. *J Clin Invest* 76:898–901, 1985
36. BONTA IL, PARNHAM MJ: Immunomodulatory-anti-inflammatory functions of E-type prostaglandins. Mini review with emphasis on macrophage-mediated effects. *Int J Immunopharmacol* 4:103–109, 1982
37. HUMES JL, BURGER S, GALAVAGE M, KUEHL FA, WIGHTMAN PD, DAHLGREN ME, DAVIES P, BONNEY RJ: The diminished production of arachidonic acid oxygenation products by elicited mouse peritoneal macrophages: Possible mechanisms. *J Immunol* 124: 2110–2116, 1980
38. SUTTORP N, SEEGER W, ZUCKER-REIMANN J, ROKA L, BHAKDI S: Mechanism of leukotriene generation in polymorphonuclear leukocytes by Staphylococcal alpha-toxin. *Infect Immun* 55:104–110, 1987